PRELIMINARY AMENDMENT TO THE SPECIFICATION

In the Specification:

Please replace paragraph [0001] of the specification with the following replacement paragraph [0001], marked to show the change made.

> [0001] This application is a national stage application of International Application No. PCT/US2004/041640, filed December 13, 2004, which claims priority to U.S. Application No.: 10/734,998, filed December 12, 2003. This application is related to U.S. Application No. 10/124,654, filed on April 16, 2002, which is a continuation-in-part of U.S. Application No. 09/929,275, filed on August 13, 2001, and of U.S. Application No. 10/008,482, filed on November 13, 2001, the latter of which is a continuation of U.S. Application No. 09/507,707, filed on February 18, 2000. This application is also related to U.S. Application No. 10/674,652, filed on September 29, 2003, which is a continuation of the aforementioned U.S. Application No. 10/008,482, which is a continuation of aforementioned U.S. Application No. 09/507,707. This application is further related to U.S. Application No. 09/978,515, filed on October 15, 2001, which is a continuation-in-part of the aforementioned U.S. Application No. 09/929,275. Each of the foregoing applications is incorporated herein in its entirety by this reference.

Please replace paragraph [0014] of the specification with the following replacement paragraph [0014], marked to show the change made.

> [0014] **Figure 1** is a schematic representation of a synthesis of a photopolymerized ~~sol-sel~~ sol-gel material, or monolith.

Please replace paragraph [0031] of the specification with the following replacement paragraph [0031], marked to show the change made.

[0031] When the linker comprises an aldehyde functional group, the amine of the enzyme and the aldehyde on the PSG surface react at low temperature, such as from about 4°C to about 23°C, or room temperature (about 20°C to about 25°C), to form an imine bond, typically in a period of from about 30 to about 60 minutes. This reaction is associated with Schiff chemistry. When the linker comprises a succinimide functional group, the amine of the enzyme and the succinimide on the PSG surface readily react at this low temperature. By way of example, the reaction may take place in a relatively short period of from about 30 to about 60 minutes at a temperature of from about 4°C to about 23°C, or room temperature (from about 20°C to about 25°C). Use of a linker with a succinimide functional group may be advantageous in that the resulting PSG-bound enzyme is less susceptible to water hydrolysis than is a PSG-bound enzyme resulting from Schiff chemistry. Regardless of the type of derivatization reaction employed, the reaction between an enzyme and the derivatized PSG material results in the binding of the enzyme to the PSG surface, preferably via enzyme-immobilizing covalent ~~bounds~~ bonds.

Please replace paragraph [0035] of the specification with the following replacement paragraph [0035], marked to show the changes made.

[0035] According to yet another aspect of the invention, a device comprising an immobilized-enzyme microreactor in a micropipet tip is provided. This microreactor is prepared by introducing a volume of a sol-gel reaction solution, as further described herein, into a micropipet tip prior to a photopolymerization reaction. The length of the microreactor is controlled by this volume of solution. The solution is then ~~photo-polymerized~~ photopolymerized to form a PSG material and

an enzyme is bound to a surface of the PSG material using a procedure similar to that described above.

Please replace paragraph [0038] of the specification with the following replacement paragraph [0038], marked to show the changes made.

[0038] A schematic representation of a synthesis of a PSG material or monolith is shown in **Figure 1**. The metalorganic monomer may comprise a metal alkoxide, such as a silane, or a mixture of metal alkoxides. Merely by way of example, the metalorganic monomer shown in **Figure 1** is methacryloxypropyltrimethoxysilane. The metal of the metalorganic monomer may comprise any of, or any combination of, the following metals or metalloids: aluminum, barium, antimony, calcium, chromium, copper, erbium, germanium, iron, lead, lithium, ~~phosphoros~~ phosphorus, potassium, silicon, tantalum, tin, titanium, vanadium, zinc, and zirconium. The foregoing list of possible metals or metalloids is merely illustrative, not limiting.

Please replace paragraph [0039] of the specification with the following replacement paragraph [0039], marked to show the changes made.

[0039] A suitable metalorganic monomer may also comprise a photoactive group or a non-hydrolyzable photoactive group, such as a methacrylate group. An example of such a metalorganic monomer is trimethoxysilypropyl methacrylate, also known as ~~methacryl-oxypropyltrimethoxysilane~~ methacryloxypropyltrimethoxysilane, as shown in **Figure 1**. Further, such a metalorganic monomer may be combined with another metalorganic monomer, as exemplified by a combination of methacryloxypropyltrimethoxysilane and bis(triethoxysilyl)ethane or bis(triethoxysilyl)octane, for example.

Please replace paragraph [0045] of the specification with the following replacement paragraph [0045], marked to show the changes made.

[0045] After the above-described combination has been partially condensed, it is polymerized to form a porous matrix. Preferably, the combination is photopolymerized, as shown in **Figure 1** by way of example. In a photopolymerization process, the combination is irradiated with suitable radiation such that the photoactive group and/or the photoinitiator absorbs the radiation from the radiation source. The ~~photo-polymerization~~ photopolymerization process may take on the order of 5 minutes, as shown in **Figure 1**, merely by way of example. The radiation may be visible or ultraviolet light. The wavelength of the radiation is selected in accordance with the type of photoactive group and/or photoinitiator that is used. By way of example, a methacrylate photoactive group may be photopolymerized at a wavelength of about 300 nm to about 365 nm, as reported in C. Yu *et al.*, *Electrophoresis* 2000, *21*, (1) 120-127 and H.G. Woo *et al.*, *Bull. Korean Chem. Soc.* 1995, *16*, 1056-1059. Further by way of example, the Irgacure 1800 photoinitiator may be photopolymerized at a wavelength of about 365 nm. Absorption of the radiation starts a photochemical reaction that catalyzes the polymerization of the metalorganic monomer, and/or associated dimers, trimers, and/or oligomers. The photopolymerization results in the formation of a relatively homogeneous porous matrix. An example of a product of a photopolymerization process is shown in **Figure 1**.

Please replace paragraph [0056] of the specification with the following replacement paragraph [0056], marked to show the change made.

[0056] In the enzyme-bonding process, the PSG material is conditioned with a conditioning agent, such as a buffer solution that will not interfere with the bioactivity of the enzyme. An example of a suitable buffer solution is a phosphate-buffered saline (PBS) solution

(about 50 mM, for example), as shown in **Figure 4** by way of example, that contains a high concentration of calcium chloride (about 200 mM, for example) to prevent self-destruction of the enzyme. A dilute enzyme-buffer solution (about 10 mM to about 100 mM, for example) is applied to the PSG material and allowed to flow through the PSG material. An example employing a trypsin enzyme, and resulting in an immobilized-enzyme PSG material, is shown in **Figure 4** by way of example.

Please replace paragraph [0057] of the specification with the following replacement paragraph [0057], marked to show the change made.

[0057] A derivatized PSG material **40** may be incorporated into a microreactor device, such as the integrated microreactor-column **10** **(Figure 2)** or the integrated microreactor-micropipet **20** **(Figure 3)** described above. When a derivatized PSG material **40** is used in a microreactor device, the ends of the device are sealed, for example, using sealing devices, and the reaction between the amine group of the enzyme and the aldehyde group at the surface of the PSG material is allowed to proceed at a low temperature, such as from about 4°C to about 40°C for several hours, such as from 30 minutes to about 24 hours, such as from about 15 hours to about 24 hours. Higher temperatures may be used to shorten the reaction period, although care should be taken to avoid temperatures that result in the autolysing, self-destruction, or degradation of the enzyme. Appropriate temperatures generally depend on the nature of the enzyme. The microreactor device is then unsealed, for example, by removing any sealing devices, and any unbound trypsin is removed from the device by flowing copious amounts of phosphate-buffered saline through the PSG material.

Please replace paragraph [0059] of the specification with the following replacement paragraph [0059], marked to show the change made.

[0059] Additionally, it will be understood that any means of immobilizing the enzyme relative to the PSG material, and any combination of such means, are contemplated herein. As mentioned above, a flow-through technique, wherein an enzyme solution ~~is~~ flows through a PSG material and the immobilization is allowed to proceed at a low temperature for a number of hours, is one example. Merely by way of example, a further example is a pump-through technique, wherein an enzyme solution is pumped through a PSG material and the immobilization is allowed to proceed at a low temperature for a suitable time, such as a number of hours. Yet another example is incubation, such as incubation of a PSG material in an enzyme solution at an appropriate temperature for an appropriate time, such as about 4°C to about 40°C for about 30 minutes to about 24 hours, such as about 15 hours to about 24 hours.

Please replace paragraph [0066] of the specification with the following replacement paragraph [0066], marked to show the change made.

[0066] Reagents and Chemicals. All buffers and solutions were prepared using a Milli-Q water purification system from Millipore (Billerica, Massachusetts) and degassing by sonication for 5 minutes prior to use. Methacryloxypropyltrimethoxysilane (MPTMS), polyethylene glycol di-methacrylate with an average molecular weight of 330 Daltons (PEG-DM), toluene, N_{α}-benzoyl-L-arginine ethyl ester (BAEE), N_{α}-benzoyl-L-arginine (BA), bradykinin, and phosphate-buffered saline (PBS) were purchased from Sigma-Aldrich (Milwaukee, Wisconsin) and used as received. BAEE, BA, and bradykinin were all dissolved in 50 mM TRIS-HCl (pH 7.4 or 7.5) buffer for analysis. Irgacure 1800 was received from Ciba, Inc. (~~Tarry-town~~ Tarrytown, New York, USA).

Please replace paragraph [0088] of the specification with the following replacement paragraph [0088], marked to show the change made.

> [0088] The present invention provides an enzyme that is immobilized by covalent bonding to the surface of a porous PSG material that is versatile in application. For example, the immobilized-enzyme, porous PSG material, or monolith, can be used in a variety of formats, such as a microreactor, a capillary or a micropipet format, as well as other formats. The immobilized-enzyme, porous PSG material provides a suitable, if not ideal, interface for downstream analysis applications and instrumentation, such as a variety of separation applications and tools including HPLC, CEC, and CE, for example. In another example, the immobilized-enzyme, porous PSG material provides an interface for separation by mass spectrometry, wherein the reaction products of the enzymatic digestion process are fed into a mass spectrometer using an electrospray ionization interface, for example.

Please replace paragraph [0089] of the specification with the following replacement paragraph [0089], marked to show the change made.

> [0089] The immobilized-enzyme, porous PSG material is particularly useful in the separation of proteinaceous and/or peptidic analytes from sample, and may be designed to accommodate such analytes that are relatively large. In a particular application, the bioactivity of the enzyme, trypsin, was enhanced up to 200-fold by being immobilized onto a PSG material, even when the temperature employed was less than the optimal temperature of the activity of that enzyme. Such an immobilized-enzyme, PSG material is thus particularly useful in biomolecule separations, such as separations of one or more proteins, oligonucleotides, peptides, steroids, organic acids, or any combination thereof.

Please replace paragraph [0090] of the specification with the following replacement paragraph [0090], marked to show the change made.

> [0090] Immobilization of an enzyme with respect to a PSG material, as described herein, can result in an increase in the activity of the enzyme by ~~a~~ several or many orders of magnitude. It is believed, without being so bound, that in the immobilization process, the enzyme becomes covalently bound to the surfaces in the pores of the PSG material. When the analyte, or substrate, enters a pore, its concentration in the pore relative to its concentration in the bulk of the material, is greatly increased. As the activity of the enzyme depends on the substrate concentration, typically in direct proportion, it too is greatly increased. As such, the immobilized-enzyme, PSG material of the present invention provides a highly active and effective tool for the breakdown, separation, and analysis of biomolecules and their components.

Please replace the abstract with the replacement abstract, marked to show the changes made, that appears on a separate sheet.